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(54) Title: MULTI-COMPONENT VACCINE COMPRISING AT LEAST THREE ANTIGENS TO PROTECT AGAINST DISEASE CAUSED BY *HAEMOPHILUS INFLUENZAE*

(57) Abstract

A multi-component immunogenic composition confers protection on an immunized host against infection caused by *Haemophilus influenzae*. Such composition comprises at least three different antigens of *Haemophilus influenzae*, two of which are adhesins. High molecular weight (HMW) proteins and *Haemophilus influenzae* adhesin (Hia) proteins of non-typeable *Haemophilus influenzae* comprise the adhesin components while the other antigen is a non-proteolytic analog of Hin47 protein. Each component does not impair the immunogenicity of the others. The *Haemophilus* vaccine may be combined with DTP component vaccines, which may contain inactivated poliovirus, including type 1, type 2 and/or type 3, and/or a conjugate of a capsular polysaccharide of *Haemophilus influenzae* and tetanus or diphtheria toxoid, including PRP-T, to provide a multi-valent component vaccine without impairment of the immunogenic properties of the other antigens.

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has been described in copending United States Patent Application No. 09/167,568 filed October 7, 1998, assigned to the assignee hereof and the disclosure of which is incorporated herein by reference. A chinchilla nasopharyngeal colonization model has been developed specifically to demonstrate vaccine efficacy of adhesins (ref. 14) and the rHMW proteins are protective in this model as described in the aforementioned copending US Patent Application No. 09/167,568. The rHMW1A and rHMW2A proteins were shown to afford equivalent protection and the rHMW1A protein was chosen for further vaccine studies. In this application, rHMW refers to recombinant HMW1A from NTHi strain 12, although other corresponding recombinant HMW1A proteins from other NTHi strains and corresponding HMW2A proteins from NTHi strains may be employed. The corresponding naturally-occurring proteins may be employed.

A second family of high molecular weight adhesion proteins has been identified in about 25% of NTHI and in encapsulated *H. influenzae* strains (refs. 15, 16, 17). The NTHi member of this second family is termed *Haemophilus influenzae* adhesin or Hia and the homologous protein found in encapsulated strains is termed *Haemophilus influenzae* surface fibril protein or Hsf.

U.S. Patent No. 5,646,259 (St. Geme, III et al), assigned to St. Louis University and Washington University, and the disclosure of which is incorporated herein by reference, describes the cloning, expression and sequences of genes encoding the Hia and Hsf proteins, which have limited homology to the HMW1 and HMW2 proteins of USP 5,603,938.

The *hia* gene was originally cloned from an expression library using convalescent sera from an otitis media patient, which indicates that it is an important immunogen during disease. The prototype Hia and Hsf proteins demonstrate about 82% sequence similarity, although the Hsf protein is considerably larger. The proteins are comprised of conserved amino and carboxy termini and several repeat motifs, with Hsf containing more repeat sequences than Hia.

United States Patent Application No. 09/268,347 filed March 16, 1999, assigned to the assignee hereof and the disclosure of which is incorporated herein by reference, describes the production of full-length and N-terminal truncated

versions of the Hia protein (rHia) in *E. coli*. These recombinant proteins have been demonstrated to protect against bacteremia caused by *H. influenzae* type a and type b organisms, and to confer partial protection against nasopharyngeal colonization by non-typeable *H. influenzae*. In this application, rHia refers to V38 rHia from NTHi strain 11, although other recombinant full-length and N-terminal truncated Hia proteins from other NTHi strains may be employed. The corresponding naturally-occurring proteins may be employed.

When under environmental stress, such as high temperature, organisms overproduce stress response or heat shock proteins (hsps). Bacterial hsps have been shown to be important immunogens, stimulating both B cells and T cells (ref. 18). The bacterial HtrA or DegP heat shock proteins are expressed under conditions of stress and the *H. influenzae* HtrA or Hin47 protein has been shown to be a partially protective antigen in the intrabulla challenge model of otitis media (ref. 19). The HtrA proteins are serine proteases and their proteolytic activity makes them unstable. In addition, as components of a multicomponent vaccine, the wild-type HtrA protein will degrade mixed antigens. The site-directed mutagenesis of the *H. influenzae htrA* gene (termed *hin47*) and the properties of the mutants have been fully described in U.S. Patent No. 5,506,159 (Loosmore et al), assigned to the assignee hereof and the disclosure of which is incorporated herein by reference.

US Patent No. 5,506,139 (Loosmore et al) describes the preparation of analogs of *Haemophilus influenzae* Hin47 protein which have a decreased protease activity which is less than about 10% of that of the natural Hin47 protein and which preferably have substantially the same immunogenic properties as natural Hin47 protein. The patent also describes the isolation, purification and characterization of nucleic acid molecules encoding the Hin47 analogs. The natural Hin47 protein is immunologically conserved among non-typeable and type b isolates of *H. influenzae*. The amino acid sequence of the natural Hin47 protein and the nucleotide sequence of the encoding *hin47* gene are described in WO 94/00149 published January 6, 1994 and incorporated herein by reference.

The Hin47 analogs of US Patent No. 5,506,139 are prepared by deleting or replacing by a different amino acid at least one amino acid of the natural Hin47

June 16, 1994 and 08/483,856 filed June 7, 1995, assigned to the assignee hereof and the disclosures of which are incorporated herein by reference (WO 95/34308, published November 21, 1995). The adjuvant preferably may comprise aluminum phosphate or aluminum hydroxide (collectively known as alum).

The components of the immunogenic composition may be present in appropriate quantities to provide the desired immune response. The components may be formulated as a vaccine for *in vivo* administration to the host. The vaccine composition may contain:

(a) about 25 to 100 μg of the Hin47 protien,

(b) about 25 to 100 μg of the Hia protien, and

(c) about 25 to 100 μg of the HMW protien.

The immunogenic compositions may be formulated with other antigenic components to provide a multivalent vaccine in which the additional antigenic component(s) confer protection against disease caused by another pathogen(s). Such additional antigens should be such that and should be present in quantities such that the immunogenicity of the individual components of the resulting vaccine is not impaired by other individual components of the composition. Such additional antigens preferably are purified antigens in defined quantities to provide a component vaccine.

Such additional antigens may be those traditionally found in multivalent protective vaccines, such as diphtheria toxoid, tetanus toxoid and pertussis antigens, including pertussis toxoid, filamentous hemagglutinin, pertactin and/or agglutinogens.

The resulting multivalent vaccine also may contain non-virulent poliovirus, such as inactivated poliovirus, which may be type 1, type 2 and/or type 3 poliovirus. The multi-component vaccine further may comprise a conjugate of a tetanus or diphtheria toxoid and a capsular polysaccharide of *Haemophilus influenzae*, preferably PRP-T.

The invention extends to a method of immunizing a host against disease caused by infection by *Haemophilus influenzae*, including otitis media, which comprises administering to the host an immunoeffective amount of the immunogenic composition provided herein.

GENERAL DESCRIPTION OF THE INVENTION

The production and purification of recombinant *H. influenzae* antigens rHMW, rHia and H91A Hin47 have been fully described in the aforementioned US Patent Applications Nos. 09/167,568, 09/268,347 and the aforementioned US Patent No. 5,506,159, respectively.

Colonization of the nasopharynx is the first step in disease development for many bacterial or viral pathogens and vaccines containing adhesin molecules should protect against this first step in disease progression. The high molecular weight (HMW) proteins, found in approximately 75% of non-typeable *H. influenzae*, have been shown to be adhesins that are protective against colonization when administered in a vaccine composition. The HMW proteins are not present in encapsulated *H.influenzae* strains or in about 25% of non-typeable *H. influenzae* strains, thus they are not sufficient for a fully-effective vaccine having strain-wide protectivity.

The Hia/Hsf proteins also have been shown to be adhesins and are present in all encapsulated *H. influenzae* strains and in most of those non-typeable *H. influenzae* strains which do not produce HMW proteins. The rHia protein is protective against colonization by NTHi and against bacteremia caused by *H. influenzae* type a and type b organisms. There is a small percentage of NTHi strains that produce neither HMW nor Hia proteins.

The HtrA protein or Hin47 is found in all encapsulated and non-typeable *H. influenzae* strains. Hin47, or its non-proteolytic H91A Hin47 mutant, is protective against bacteremia caused by *H. influenzae* type b and otitis media caused by non-typeable *H. influenzae*, but it does not prevent colonization. A combination vaccine comprising rHMW, rHia and H91A Hin47 antigens may be formulated to protect against *H. influenzae* disease, including otitis media. Such combination is provided herein.

The composition of multi-component vaccines is critical for maximum efficiency. The vaccine components must be compatible and they must be combined in appropriate ratios to avoid antigenic interference and optimize any possible synergies. If administered with other established vaccines, they must not interfere with the protection afforded by the vaccine against other disease(s).

The preparation, immunogenic and protective properties of a two-component rHMW + H91A Hin47 vaccine have been described in US Patent Application No. 09/210,995 filed December 15, 1998, assigned to the assignee hereof and the disclosure of which is incorporated herein by reference.

Various antigen ratios were compared for the three component H91A Hin47 + rHMW + rHia vaccine, in two animal species. There was no affect on the anti-H91A Hin47 response with increasing amounts of rHia. Antigenic interference was observed in mice for the anti-rHMW response, when a 0.3 μg dose of each of H91A Hin47 + rHMW was mixed with increasing doses of rHia. However, at a 3.0 μg dose of each of H91A Hin47 + rHMW, there was no suppression of the anti-rHMW response with increasing amounts of rHia. Although there was a transient suppression of the anti-Hia response on day 42 when a 0.3 μg dose was combined with 3 μg each of H91A + rHMW, this effect was not significant by day 56. In guinea pigs, the anti-H91A Hin47 and anti-rHMW responses were not effected by the addition of rHia. However, there appeared to be a small, but statistical, effect on the anti-Hia response in the presence of H91A Hin47 + rHMW for the booster immunizations. These data indicate that the composition of the three component vaccine is critical to achieve a good immune response to all components.

Referring to Fig. 1, there is illustrated the antibody response in mice to the H91A Hin47 antigen of a three-component H91A Hin47 + rHMW + rHia vaccine. High antibody titers were achieved with all vaccine combinations at the final bleed. Referring to Fig. 2, there is illustrated the antibody response in mice to the rHMW antigen of a three-component H91A Hin47 + rHMW + rHia vaccine. At the 3.0 μg dose of each of H91A Hin47 + rHMW, there are high titers of anti-rHMW antibodies found in the final bleed sera irrespective of the amount of rHia in the vaccine composition. However, at the 0.3 μg dose of each of H91A Hin47 + rHMW, the anti-rHMW titers are dramatically reduced with increasing amounts of rHia added. Referring to Fig. 3, at the 0.3 μg dose of rHia, there is a suppressive effect on the anti-rHia immune response on day 42 with increasing amounts of H91A Hin47 + rHMW. However, this effect is lost by day 56 and is not observed with higher doses, where there is no consequence on the immune response.

and the cells were resuspended in 1.5 mL of 10% glycerol, aliquotted as 40 µl samples, and stored at -70°C.

One aliquot of electrocompetent BL21(DE3) cells was thawed on ice and approximately 9 ng of DS-2150-1 DNA was added. Samples were incubated on ice for 3 min. then transferred to a -20°C BioRad Gene Pulser electrode cuvette and subjected to an electric pulse. 900 µl of SOC medium were added and the mixture transferred to a culture tube where it was incubated at 37°C for 1 hour before being plated onto YT agar containing 25 µg/mL kanamycin. The plate was incubated overnight at 37°C and single colonies were used for expression studies.

Individual clones were grown in NZCYM medium to an $A_{600\ nm}$ of approximately 0.3 and lactose was added to 1% to induce expression. Cells were grown for 4 hours, then harvested and analysed by SDS PAGE. Clone DS-2171-1-1 was chosen as a representative clone which expressed high levels of H91A Hin47.

The *E. coli* containing DS-2171-1-1 was grown in 2 X 2 L flasks containing 250 mL of ECGM (containing 8 g/L glucose, pH 6.5) and incubated by shaking at 37°C for approximately 9 hours in the dark at 250 rpm. The culture fluid (2 x 250 mL) was inoculated into a 10 L fermentor and the culture grown at 37°C. After approximately 10 hours of incubation, 1% lactose (final concentration) is added for induction, followed by an additional 4 hours incubation.

The culture fluid was harvested into sterile transfer bottles and concentrated and diafiltered by cross-flow filtration against 50 mM Tris/HCl buffer, pH 8.0. The cells in the concentrate are lysed using a high-pressure homogenizer (2 passes at 15,000 psi) to release the H91A Hin47 protein. The cell debris was removed by centrifugation at 15,000 rpm for 1.5 hours. The supernatant was further clarified by centrifugation and filtered through a 0.22 µm dead-end filter. Products may be stored frozen at -70°C until further processing.

Sodium chloride (NaCl) was added to the clarified sample to a final concentration of 100 mM. The sample was then purified on an anion exchange chromatography column (TMAE-Fractogel) equilibrated with 50 mM Tris pH 8.0

containing 100 mM NaCl. The H91A Hin47 protein was obtained in the run-through.

The aqueous layer was loaded onto a ceramic hydroxyapatite type 1 (CHTP-1) column equilibrated with 10 mM sodium phosphate buffer pH 8.0. The column was then washed with 150 mM sodium phosphate buffer pH 8.0 and H91A Hin47 was eluted with 175 mM sodium phosphate buffer, pH 8.0 containing 1 M NaCl.

The H91A Hin47 purified protein was concentrated using a 10 kDa molecular weight cut-off membrane followed by diafiltration with approximately 10 volumes of phosphate buffered saline (PBS), pH 7.5.

The H91A Hin47 purified protein in PBS was passed through a Q600 Sartobind membrane adsorber. After passing the solution, the membrane was regenerated using 1.0 M KCl/1.0 M NaOH followed by washing with 1 M KCl then equilibrating with PBS. The process was repeated twice. The concentrated diafiltered H91A Hin47 protein was sterile filtered through a 0.22 μm membrane filter. Sterile H91A Hin47 protein was adjuvanted with aluminum phosphate. The adosrbed purified concentrate was diluted to produce the adsorbed bulk at 100 μg/mL.

The concentration of the H91A Hin47 vaccine component was adjusted to 400 $\mu g\ ml^{-1}$ in PBS (pH 7.3) and was adjuvanted with aluminum phosphate to a final concentration of 3 $mg\ ml^{-1}$. Different doses were prepared by diluting the stock with 3 $mg\ ml^{-1}$ of aluminum phosphate in PBS.

Example 2

This Example describes the preparation of a rHMW vaccine component.

The production and purification of the rHMW protein has been described in the aforementioned copending United States Patent Application No. 09/167,568 filed October 7, 1998 and is shown schematically in Figure 9.

Briefly, plasmid pHMW1-15 (ref. 13) contains a *Xba* I site within the T7 promoter sequence and a unique *BamH* I site within the coding sequence of the mature HMW1A protein of non-typeable *Haemophilus* strain 12. The 1.8 kb *Xba* I-*BamH* I fragment of pHMW1-15 was deleted and replaced by an approximately 114 bp *Xba* I-*BamH* I fragment generated from oligonucleotides. The resultant 11.3 kb

plasmid, DS-1046-1-1, thus contains the T7 promoter joined in frame with the *hmw1ABC* operon that encodes the mature 125 kDa HMW1A protein (Fig. 9).

Plasmid DS-1046-1-1 contains the *T7 hmw1ABC* gene cassette and has a unique *Bgl* II site outside the coding region of the mature HMW1A gene. Plasmid DS-2224-1-4 contains the *E. coli cer* gene located on a *Bam*H I fragment. This fragment was isolated and ligated into the *Bgl* II site of plasmid DS-1046-1-1 to produce plasmid BK-35-4 (Fig. 9). The kanamycin resistance cassette was excised from pUC 4K by *Sal* I restriction and ligated into the *Sal* I restricted BK-35-4 plasmid to produce plasmid BK-76-1-1.

Plasmids were introduced into *E. coli* BL21(DE3) cells by electroporation using a BioRad apparatus. Strains were grown at 37°C in NZCYM medium to an optical density of A_{578}=0.3, then induced by the addition of lactose to 1.0% for 4 hours. Samples were adjusted to 0.2 OD/μl with SDS-PAGE lysis + loading buffer and the same amount of protein sample was loaded onto SDS-PAGE gels. Clone BK-116-1-1 was selected as a representative clone for preparation of seed stocks.

Recombinant HMW protein was expressed as inclusion bodies in *E. coli*, and were purified by the same procedure (Figure 12) *E. coli* cell pellets from 500 ml culture were resuspended in 50 ml of 50 mM Tris-HCl, pH 8.0, containing 0.1 M NaCl, and disrupted by sonication. The extract was centrifuged at 20,000 *g* for 30 min and the resultant supernatant was discarded. The pellet was further extracted, in 50 ml of 50 mM Tris-HCl, pH 8.0 containing 0.5% Triton X-100 and 10 mM EDTA, then centrifuged at 20,000 *g* for 30 min, and the supernatant was discarded. The pellet was further extracted in 50 ml of 50 mM Tris-HCl, pH 8.0, containing 1 % octylglucoside, then centrifuged at 20,000 *g* for 30 min, and the supernatant was discarded.

The resultant pellet, obtained after the above extractions, contains the inclusion bodies. The pellet was solubilized in 6 ml of 50 mM Tris-HCl, pH 8.0, containing 6 M guanidine and 5 mM DTT. Twelve ml of 50 mM Tris-HCl, pH 8.0 was added to this solution and the mixture was centrifuged at 20,000 *g* for 30 min. The supernatant was precipitated with polyethylene glycol (PEG) 4000 at a final concentration of 7%. The resultant pellet was removed by centrifugation at 20,000 *g* for 30 min and the supernatant was precipitated by $(NH_4)_2SO_4$ at 50%

saturation. After the addition of $(NH_4)_2SO_4$, the solution underwent phase separation with protein going to the upper phase, which was then subjected to centrifugation at 20,000 *g* for 30 min. The resultant pellet was dissolved in 2 ml of 50 mM Tris-HCl, pH 8.0, containing 6 M guanidine HCl and 5 mM DTT and the clear solution was purified on a Superdex 200 gel filtration column equilibrated in 50 mM Tris-HCl, pH 8.0, containing 2 M guanidine HCl. The fractions were analysed by SDS-PAGE and those containing the purified rHMW1 were pooled and dialysed overnight at 4°C against PBS, then centrifuged at 20,000 *g* for 30 min. The protein remained soluble under these conditions and glycerol was added to the rHMW1 preparation at a final concentration of 20% for storage at -20°C.

The concentration of the rHMW vaccine component was adjusted to 400 $\mu g\ ml^{-1}$ in PBS (pH 7.3) and was adjuvanted with aluminum phosphate to a final concentration of 3 $mg\ ml^{-1}$. Different doses were prepared by diluting the stock with 3 $mg\ ml^{-1}$ aluminum phosphate in PBS.

Example 3

This Example illustrates the preparation of a rHia vaccine component.

The production and purification of the rHia protein has been described in the aforementioned copending United States Patent Application No. 09/268,347 filed March 16, 1999 and is shown schematically in Figure 10.

Briefly, chromosomal DNA was purified from NTHi strain 11 and the full-length *hia* gene was *PCR* amplified using the oligonucleotides (5038.SL and 5039.SL) (Fig. 11). The PCR product contained an NdeI site at the 5' end and a BamHI dite a the 3' end. This fragment was cloned into the NdeI/BamHI restricted pT7-7 expression vector (ref.20)producing plasmid DS-2008-2-3 (Fig. 10).

PCR primers (5526.SL and 5528.SL) (Fig 12) were used to amplify a truncated *hia* gene fragment from the V38 site to the Sty I site of plasmid DS-2008-2-3, the resulting fragment was TA cloned into plasmid pCRII (Invitrogen) to produce plasmid DS-2153-3-5. This plasmid was then restricted with Nde I and Sty I and this fragment was ligated to the Nde I/Sty I 5.7kb isolated vector fragment from DS-2008-2-3 to produce plasmid DS-2186-2-1.

Plasmid DS-2186-2-1 containing the V38 truncated *hia* gene, was restricted with Bgl II and BamH I to release the *rHia* gene. This fragment was isolated and cloned into the BglII restricted, CAP treated, plasmid BK-2-1-2, to produce plasmid BK 96-2-11. This plasmid now possesses a kanamycin resistance marker and the *E. coli cer* gene as well as the truncated V38 strain 11 *hia* gene.

The concentration of the rHia vaccine component was adjusted to 400 μg ml^{-1} in PBS (pH 7.3) and was adjuvanted with aluminum phosphate to a final concentration of 3 mg ml^{-1}. Different doses were prepared by diluting the stock with 3 mg ml^{-1} aluminum phosphate in PBS.

Example 4

This Example describes the combination of H91A Hin47 + rHMW + rHia as a three-component vaccine.

The preparation of a two-component vaccine comprising H91A Hin47 + rHMW, has been described in the aforementioned copending United States Patent Application No. 09/210,995 filed December 15, 1998. Briefly, vaccines were prepared that comprised combination of H91A Hin47 and rHMW by combining components on day 0, mixing overnight at 4°C and aliquotted on day 1. The combined vaccines were stored at 4°C throughout the immunization period.

Vaccines were prepared that comprised the following combinations of rHia with the two component vaccine contained in Table II:

TABLE II

rHia→ 2 COMPONENT ↓	0	0.3	1.0	3.0	10	25	50	100
0		m	m	m	m	gp	gp	gp
0.3 + 0.3	m	m	m	m	m			
3.0 + 3.0	m	m	m	m	m			
25 + 25	gp					gp	gp	gp
50 + 50	gp					gp	gp	gp

Notes: 2 component refers to H91A Hin47 + rHMW

m indicates the vaccine was used to immunize mice.
gp indicates that the vaccine was used to immunize guinea pigs.

Vaccine components were combined on day 0, mixed overnight at 4°C, and aliquotted on day 1. The multi-component vaccines were stored at 4°C throughout the immunization period.

Example 5

This Example describes the analysis of the immunogenicity of the multi-component vaccines in animals.

The immunogenicity of a two-component vaccine comprising H91A Hin47 + rHMW, has been described in the aforementioned copending United States Patent Application No. 09/210,995 filed December 15, 1998.

Groups of five BALB/c mice (Charles River, Quebec) were immunized subcutaneously (s.c.) on days 1, 29 and 43 with one of the mouse vaccines described in Example 4. Blood samples were taken on days 0, 14, 28, 42, and 56.

Groups of five Hartley outbred guinea pigs (Charles River, Quebec) were immunized intramuscularly (i.m.) on days 1, 29 and 43 with one of the guinea pig vaccines described in Example 4. Blood samples were taken on days 0, 14, 28, 42, and 56.

Anti-H91A Hin47, anti-rHMW, and anti-rHia IgG antibody titers were determined by antigen specific enzyme linked immunosorbent assays (ELISAs). Microtiter wells (Nunc-MAXISORB, Nunc, Denmark) were coated with 50 μl of protein solution (0.4 μg ml^{-1} for H91A Hin47, 0.4 μg ml^{-1} for rHMW, or 0.4 μg

ml^{-1} for rHia). The secondary antibodies used were affinity-purified $F(ab')_2$ fragments of goat anti-mouse IgG (Fc-specific) or anti-guinea pig IgG (Fc-specific) antibodies conjugated to horseradish peroxidase (Jackson ImmunoResearch Labs, Mississauga, Ontario). The reactions were developed using tetramethylbenzidine (TMB/H202, ADI, Mississauga, Ontario) and absorbancies were measured at 450 nm (using 540 nm as a reference wavelength) in a Flow Multiskan MCC microplate reader (ICN Biomedicals, Mississauga, Ontario). The reactive titer of an antiserum was defined as the reciprocal of the dilution consistently showing a two-fold increase in adsorbance over that obtained with the prebleed serum sample.

The results of the immunogenicity studies are illustrated in Figures 1 to 6. As shown in Figure 1, the final bleed sera obtained from mice immunized with 0.3 ug each of H91A Hin47 + rHMW or with 3.0 μg each of H91A Hin47 + rHMW and 0, 0.3, 1.0, 3.0 or 10 μg of rHia all had equivalent high antibody titers to H91A Hin47. These data show that there is neither a synergistic nor an interfering affect on the anti-H91A Hin47 antibody titer with added rHia.

As shown in Figure 2, panel A, the final bleed sera obtained from mice immunized with 0.3 μg each of H91A Hin47 + rHMW and 0, 0.3, 1.0, 3.0 or 10 μg of rHia had significantly reduced anti-HMW titers with increased amounts of rHia added. These data indicate that at low concentrations of H91A Hin47 + rHMW, there is suppression of the anti-HMW antibody response caused by the addition of rHia. When mice are immunized with 3 μg each of H91A, Hin47 + rHMW, and 0, 0.3, 1.0, 3.0 or 10 μg of rHia, no affect was observed and high titers of anti-rHMW antibodies were obtained in the final bleed sera (Fig. 2, panel B).

As shown in Figure 3, panel A, the addition of 3.0 μg each of H91A Hin47 + rHMW to 0.3 μg of rHia, had a transient suppressive effect on the anti-rHia response at day 42, that disappeared by day 56. However, as shown in Figure 3, panels B to D, there was no suppressive effect observed at higher doses of rHia.

As shown in Figure 4, panels A and B, the final bleed sera from guinea pigs immunized with 25 μg each of H91A Hin47 + rHMW or 50 μg each of H91A Hin47 + rHMW and 0, 25, 50 or 100 μg of rHia all had high titers of anti-H91A

Hin47 antibodies. These data indicate that there was neither a synergistic nor a suppressive affect on the anti-H91A Hin47 antibody response in the presence of the three antigens.

As shown in Figure 5, panels A and B, the final bleed sera from guinea pigs immunized with 25 μg each of H91A Hin47 rHMW or 50 μg each of H91A Hin47 + rHMW and 0, 25, 50 or 100 μg of rHia all had high titers of anti-rHMW antibodies. These data indicate that there was neither a synergistic nor a suppressive affect on the anti-rHMW antibody response in the presence of the three antigens.

As shown in Figure 6, panels A to C, the final bleed sera from guinea pigs immunized with 25, 50 or 100 μg of rHia with or without added H91A Hin47 + rHMW, all had high titer anti-rHia antibodies. There was, however, a slight but statistical inhibition of the anti-rHia response after booster doses containing all three antigens.

Example 6

This Example describes the protective ability of a multi-component vaccine in animal models of disease.

In young chinchillas, it has been demonstrated that nasopharyngeal colonization with non-typeable *H. influenzae* leads to otitis media (ref. 14). rHMW is partially protective in a chinchilla nasopharyngeal colonization challenge model, as described in copending US Patent Application No. 09/167,568. In this model, animals are immunized i.m. on days 0, 14 and 28 with 25, 50 or 100 μg of rHMW, adsorbed to alum, and challenged on day 44 with 10^8 cfu of live bacteria delivered intranasally (50 μl per nares).

Nasopharyngeal lavage is performed 4 days post challenge using 1 ml of sterile saline as wash. 25 μl of wash is plated onto chocolate agar in the presence of streptomycin and the plates incubated at 37°C for 24 h. (The challenge strain was made streptomycin resistant by serial passaging, in order to facilitate the quantitation of recovered bacteria in the presence of natural flora that are killed by the streptomycin.) Convalescent animals or those mock-immunized with alum alone, are used as controls. For the multi-component vaccine study, 50 μg each of rHMW, rHia, and H91A Hin47 were mixed as described in Example 4 and

CLAIMS

1. An immunogenic composition for conferring protection in a host against disease caused by *Haemophilus influenzae*, comprising:

at least three different antigens of *Haemophilus influenzae*, at least two of which different antigens is an adhesin.

2. The immunogenic composition of claim 1 wherein one of said antigens which is an adhesin is a high molecular weight (HMW) protein of a non-typeable strain of *Haemophilus influenzae*.

3. The immunogenic composition of claim 2 wherein said HMW protein is a HMW1 or HMW2 protein of the non-typeable strain of *Haemophilus influenzae*.

4. The immunogenic composition of claim 1 wherein one of the antigens which is an adhesin is a *Haemophilus influenzae* adhesin (Hia) protein of a non-typeable strain of *Haemophilus influenzae* or a *Haemophilus influenzae* surface fibril (Hsf) protein of a typeable strain of *Haemophilus influenzae*.

5. The immunogenic composition of claim 1 wherein the antigen of *Haemophilus influenzae* which is not an adhesin is a non-proteolytic heat shock protein of a strain of *Haemophilus influenzae*.

6. The immunogenic composition of claim 6 wherein the non-proteolytic heat shock protein of a strain of *Haemophilus influenzae* is an analog of *Haemophilus influenzae* Hin47 protein having a decreased protease activity which is less than about 10% of natural Hin47 protein.

7. The immunogenic composition of claim 1, wherein one of said antigens which is at adhesin in a high molecular weight (HMW) protein of a non-typeable strain of *Haemophilus influenzae* and the other of the antigens which is an adhesin is a *Haemophilus influenzae* adhesin (Hia) protein of a non-typeable strain of *Haemophilus influenzae* or a *Haemophilus influenzae* surface fibril (Hsf) protein of a typeable strain of *Haemophilus influenzae*.

8. An immunogenic composition for conferring protection in a host against disease caused by *Haemophilus influenzae*, which comprises:

(a) an analog of *Haemophilus influenzae* Hin47 protein having a decreased protease activity which is less than about 10% of natural Hin47 protein,

Molecular Weight(kDa)		Non-typeable *H. influenzae* Strain					
		12	JoyC	K21	LCDC2	PMH1	15
Mature Protein:	HMW1	125	125.9	104.4	114.0	102.4	103.5
	HMW2	120	100.9		111.7	103.9	121.9

21. The composition of claim 8 further comprising an adjuvant.

22. The composition of claim 21 wherein said adjuvant is aluminum hydroxide or aluminum phosphate.

23. The composition of claim 8 comprising

(a) about 25 to about 100 μg of the Hin47 protein analog, and

(b) about 25 to about 100 μg of the Hia protein, and

(c) about 25 to about 100 μg of the HMW protein.

24. The composition of claim 8 further comprising at least one additional antigenic component for conferring protection against infection caused by another pathogen.

25. The composition of claim 8 wherein said at least one additional antigenic component is selected from the group consisting of diphtheria toxoid, tetanus toxoid, pertussis antigens, non-virulent poliovirus and PRP-T.

26. The composition of claim 25 wherein said pertussis antigens are selected from the group consisting of pertussis toxoid, filamentous hemagglutinin, pertactin and agglutinogens.

27. A method of immunizing a host against disease caused by infection with *Haemophilus influenzae*, including otitis media, which comprises administering to the host an immunoeffective amount of a composition as claimed in claim 1 or 8.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

(88) Date of publication of the international search report:
25 January 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/51633 A3

(54) Title: MULTI-COMPONENT VACCINE COMPRISING AT LEAST THREE ANTIGENS TO PROTECT AGAINST DISEASE CAUSED BY *HAEMOPHILUS INFLUENZAE*

(57) Abstract: A multi-component immunogenic composition confers protection on an immunized host against infection caused by *Haemophilus influenzae*. Such composition comprises at least three different antigens of *Haemophilus influenzae*, two of which are adhesins. High molecular weight (HMW) proteins and *Haemophilus influenzae* adhesin (Hia) proteins of non-typeable *Haemophilus influenzae* comprise the adhesin components while the other antigen is a non-proteolytic analog of Hin47 protein. Each component does not impair the immunogenicity of the others. The *Haemophilus* vaccine may be combined with DTP component vaccines, which may contain inactivated poliovirus, including type 1, type 2 and/or type 3, and/or a conjugate of a capsular polysaccharide of *Haemophilus influenzae* and tetanus or diphtheria toxoid, including PRP-T, to provide a multi-valent component vaccine without impairment of the immunogenic properties of the other antigens.

INTERNATIONAL SEARCH REPORT

Interr Application No
PCT/CA 00/00207

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/102 A61K39/116 A61K39/295 A61P31/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, PAJ, WPI Data, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BARENKAMP STEPHEN J ET AL: "Identification of a second family of high-molecular-weight adhesion proteins expressed by non-typable Haemophilus influenzae." MOLECULAR MICROBIOLOGY, vol. 19, no. 6, 1996, pages 1215-1223, XP000946619 ISSN: 0950-382X cited in the application page 1215, column 2, paragraph 1 page 1220, column 2 -page 1221, column 1, paragraph 1	1-4,7-9

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 October 2000	17/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Noë, V

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	S BARENKAMP: "Immunization with high-molecular weight adhesion proteins of nontypeable H. influenzae modifies experimental otitis media in chincillas" INFECTION AND IMMUNITY,AMERICAN SOCIETY OF MICROBIOLOGY, WASHINGTON, DC,US, vol. 64, no. 4, 1996, pages 1246-1251, XP002142962 ISSN: 0019-9567 cited in the application abstract page 1250	1-3,7-9, 18,20,21
A	WO 94 21290 A (BARENKAMP STEPHEN J ;ST GEME JOSEPH WILLIAM III (US)) 29 September 1994 (1994-09-29) abstract page 2, line 1 - line 21 page 5, line 19 - line 26 page 6, line 29 - line 34 page 9, line 30 -page 10, line 9; example 1 page 23, line 7 - line 25	1-3,7-9, 18-21
A	US 5 869 302 A (LOOSMORE SHEENA M ET AL) 9 February 1999 (1999-02-09) the whole document	1,5,6, 8-15
E	WO 00 35477 A (LOOSMORE SHEENA M ;CONNAUGHT LAB (CA); YANG YAN PING (CA); KLEIN M) 22 June 2000 (2000-06-22) cited in the application the whole document	1-27

INTERNATIONAL SEARCH REPORT

ormation on patent family members

Interr Application No
PCT/CA 00/00207

Patent document cited in search report	Publicati n dat	Patent family member(s)	Publication dat
WO 9421290 A	29-09-1994	AU 696207 B	03-09-1998
		AU 6400594 A	11-10-1994
		BR 9406589 A	30-01-1996
		EP 0689453 A	03-01-1996
		JP 11501003 T	26-01-1999
		US 5869065 A	09-02-1999
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		US 6025342 A	15-02-2000
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		US 5962430 A	05-10-1999
WO 0035477 A	22-06-2000	AU 1543900 A	03-07-2000

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
1038-1023MS		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA00/00207	29/02/2000	03/03/1999
International Patent Classification (IPC) or national classification and IPC A61K39/102		
Applicant CONNAUGHT LABORATORIES LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 16 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
29/09/2000	09.05.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Herrero, M Telephone No. +49 89 2399 8542

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA00/00207

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1,2,5-7,9,10,13-15, 24-26	as originally filed	
3,4,4a,8,11,12, 16-23	with telefax of	28/02/2001

Claims, No.:

8 (part),9-19, 20 (part)	as originally filed	
1-7,8 (part),20 (part), 21-27	with telefax of	28/02/2001

Drawings, sheets:

1/19-19/19	as received on	13/07/2000	with letter of	11/07/2000

Sequence listing part of the description, pages:

1-3, filed with the letter of 18.04.00

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☒ furnished subsequently to this Authority in written form.
   - ☒ furnished subsequently to this Authority in computer readable form.

☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:

☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:
see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 27 with respect to industrial applicability.

because:

☒ the said international application, or the said claims Nos. 27 relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-27
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-27
Industrial applicability (IA)	Yes:	Claims	1-26
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

SECTION I

6. Additional observations

The Sequence Listing (i.e. information concerning SEQ ID NOs 1 to 11 on pages 1-3) subsequently filed with the letter of 18.04.00 (i.e. after the fling date of 29.02.00), does not form part of the application (Rule 13ter.1(f) PCT).

SECTION III

Claim 27 relates to medical uses considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of this claim (Article 34(4)(a)(i) PCT).

SECTION V

2. CITATIONS AND EXPLANATIONS

2.1 The following documents have been considered for the purposes of this report:

D1: Barenkamp, S.J. et al (1996) Mol. Microbiol. **19**:1215-1223 (also cited in the application)
D2: S.J. Barenkamp (1996) Infect. Immun. **64**:1246-1251 (also cited in the application)
D3: Loosmore, S. M. et al (1998) Infect. Immun. **66**:899-906 (cited in the description; identified on page 26 as reference 19)

The aforementioned D3 was not cited in the international search report. A copy of the document has been provided to the Applicants.

2.2 D1 reports the identification of the gen locus designated *hia* encoding a highly immunogenic adhesin (Hia) representative of a second family of high-molecular-

weight adhesion proteins expressed by non-typable *Haemophilus influenzae* strains that did not express HMW1/HMW2-related proteins. Identification of this second family of high-molecular-weight adhesion proteins suggests the possibility of developing vaccines based upon a combination of HMW1/HMW2-like proteins and Hia-like proteins which would be protective against disease caused by most of all non-typable *H. influenzae*. In particular D1 states that, "Although the Hia protein is expressed by only a subset of non-typable *Haemophilus influenzae* strains, its immunogenicity and role as an adhesion protein suggest its potential role as vaccine candidate. If combined with representative HMW1/HMW2-like proteins, proteins which are major non-pilus adhesins for non-typable *Haemophilus influenzae* strains that do not contain an *hia* gene, a vaccine formulation could be envisioned that would be protective against most or all non-typable *H. influenzae*" (see paragraph bridging pages 1220-1221).

D2 describes studies examining the protective potential of the *Haemophilus influenzae* high-molecular-weight adhesion proteins HMW1 and HMW2 in the chinchilla model of otitis media. The results obtained following immunization and challenge of experimental animals showed that the protection conferred by immunization with an adjuvanted mixture of HMW1/HMW2 proteins was not complete. D2 indicates that "Although an ideal vaccine should be capable of providing long-lasting and absolute protection against disease, the results with the HMW1/HMW2 proteins should still be considered encouraging" (cf page 1250, left column, last paragraph). Since the possibility of achieving full protection against nontypeable *H. influenzae* by immunization with a single purified bacterial component would appear to be doubtful, D2 proposes for this purpose to follow a strategy similar to the successful approach carried out with a bacterium such as *Bordetella pertussis,* i.e. administering a vaccine consisting of several distinct surface antigens combined in a multiple-component mixture (see page 1250, right column, last paragraph).

Document D3 reports the results of protection studies performed with two animal models (the passive infant rat model of bacteraemia and the active chinchilla model of otitis media) which demonstrate that the *Haemophilus influenzae* HtrA protein (a heat shock protein with serine protease activity) is a protective antigen. Additionally, D3 shows that the non-proteolytic HtrA analogue H91A is a protective

antigen against bacteraemia caused by *H. influenzae* type b and against otitis media caused by nontypeable *H. influenzae* (see that the *htr*A gene/HtrA protein are also referred to in the literature as *hin*47 gene/Hin47 protein). In view of this results D3 concludes that the H91A antigen may be suitable for inclusion in a multicomponent otitis media vaccine (cf sentence bridging pages 905-906). See also that in the last sentence of the discussion, D3 notes that a phase I clinical trial of the H91A HtrA protein was in progress.

2.3 Inventive step (Art 33(3) PCT)

The arguments put forward by the Applicants in their letter dated 28.02.01, in reply to the written opinion of 29.11.00, have been taken into account. Nevertheless, these arguments are not deemed to be convincing. Thus it is still considered that the application does not satisfy the criterion set forth in Art. 33(3) PCT because the subject-matter presently claimed does not involve an inventive step (Rule 65(1)(2) PCT).

The technical problem underlying the present application relates to the provision of an efficacious multicomponent vaccine suitable to provide protection against disease caused by infection with *Haemophilus influenzae*, including otitis media.

The hereby claimed solution to the problem posed basically relies on the provision of immunogenic compositions comprising at least three different antigens of *Haemophilus influenzae,* at least two of which different antigens is an adhesin and the other of which is not an adhesin. In a preferred approach the proposed immunogenic composition comprises (a) an analog of *H. influenzae* Hin 47 protein having decreased protease activity, for instance the H91A protein, (b) a *H. influenzae* adhesin Hia protein of a non-typeable strain of *H. influenzae* and (b) a high molecular weight (HMW) protein of a strain of non-typeable *H. influenzae.*

However, the aforementioned claimed solution appears to be rendered obvious by the teachings of the available prior art. In particular, the immunogenic compositions according to Claims 1-10, 16, 18, 19 and 20 cannot be regarded as inventive when considering the combination of relevant teachings derivable from D1+D3 or D3+D2 (see paragraph 2.2 above).

The generic method of immunizing a host against disease caused by infection with *H. influenzae* with the non-inventive compositions of Claims 1 or 8 according to Claim 27 is also considered as obvious, contrary to Art. 33(3) PCT, especially in view of corresponding immunization procedures carried out in D2 or D3.

Dependent Claims 11-15, 17, 21, 22, 23 and 24-26 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step for the following reasons:

Claims 11-15 identify certain advantageous analogs of Hin47 protein, devoid of undesirable protease activity, which have been previously characterized in D3 (see "Immunogenicity and protection studies" bridging pages 902-903).

Claim 17 identifies the recombinantly-produced Hia protein of interest included in the intended composition as being a N-terminal truncation V38 rHia. In the light of the supporting description it cannot be ascertain whether the experimental results described in Examples 5 and 6 correspond to the use of a three component vaccine (as depicted in Table II of page 21) comprising said V38 truncated product. Nevertheless the application as originally filed does not contain technical information showing that the use of a N-terminal V38 rHia truncated product would result in any unexpected/advantageous effect over the use of a rHia product of the type disclosed in D1. Accordingly, it is not apparent on which grounds the composition of Claim 17 should be regarded as inventive over the composition of Claim 16, which in turn has been objected to under the provisions of Art. 33(3) PCT in view of the teachings of e.g. D3+D1 (see above).

The embodiments contemplated in Claims 21 and 22 (use of alum adjuvants) are, in the present technical context, standard practice for the person skilled in the art (see e.g. the immunization procedure in D3).

No inventive contribution appears to be involved in claiming the composition according to Claim 23 insofar as the amounts of protein therein referred to merely represent normal ranges employed for the same antigens, under corresponding circumstances, in the related prior art (see e.g. D2, page 1247, left column, lines 20-22 from the bottom and D3 page 901, left column, lines 10-14).

Claims 24-26 merely recite the possible use of additional antigenic components well-known in the art which, in a desirably way, would expectedly broaden the protective value of the resulting multicomponent immunogenic composition.

2.4 Industrial applicability (Art. 33(4) PCT)

For the assessment of the present Claim 27 on the question whether it is industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claim. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

SECTION VII

The expression "hereby incorporated by reference" in respect of prior art documents on page 1, lines 19-20; page 2, lines 25-26; page 3, lines 19-20 and 31-32; page 4, lines 19-20 and 30; page 5, lines 10-11; page 8, line 2; page 12, line 4 leads to a doubt as to whether the requirements of the description being self-contained are satisfied (see PCT Guidelines C-II, 4-17).

SECTION VIII

1. Claims 9, 10 and 24 do not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. The claims attempt to define the subject-matter in terms of the result to be achieved which merely amounts to a statement of the underlying problem. The technical features necessary for achieving this result, i.e. the suitable amounts of proteins, the actual amino acid positions of interest in natural Hin47 protein and the specific additional antigenic components to be used, should have been added to Claims 9, 10 and 24, respectively.

2. The relative term "substantially" used in Claim 10 has no well-recognised meaning and leaves the reader in doubt as to the meaning of the technical features to which it refers, thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT).

3. The expression "N-terminal truncation V38 rHia" employed in Claim 17 appears to represent an internal designation which is meaningless to the skilled person, contrary to Art. 6 PCT. To overcome this deficiency, the actual technical meaning of said "N-terminal truncation V38 rHia" as presented in the supported description (cf Example 3) should have been incorporated in the claim.

4. Apparently Claim 7, line 2 was meant to read "which is an adhesin is a high molecular weight ...".

PA NT COOPERATION TREAT

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 14 November 2000 (14.11.00)	
International application No. PCT/CA00/00207	**Applicant's or agent's file reference** 1038-1023MIS
International filing date (day/month/year) 29 February 2000 (29.02.00)	**Priority date** (day/month/year) 03 March 1999 (03.03.99)
Applicant LOOSMORE, Sheena, M. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

29 September 2000 (29.09.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Manu Berrod

Telephone No.: (41-22) 338.83.38

PA ENT COOPERATION TREA

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

STEWART, Michael, I.
Sim & McBurney
6th Floor
330 University Avenue
Toronto, Ontario M5G 1R7
CANADA

Date of mailing (day/month/year)
03 August 2001 (03.08.01)

Applicant's or agent's file reference
1038-1023MIS:sd

IMPORTANT NOTIFICATION

International application No.
PCT/CA00/00207

International filing date (day/month/year)
29 February 2000 (29.02.00)

1. The following indications appeared on record concerning:
[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address
CONNAUGHT LABORATORIES LIMITED
1755 Steeles Avenue West
Toronto, Ontario M2R 3T4
Canada

State of Nationality: CA
State of Residence: CA
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:
[ ] the person [X] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address
AVENTIS PASTEUR LIMITED
1755 Steeles Avenue West
Toronto, Ontario M2R 3T4
Canada

State of Nationality:
State of Residence:
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:
[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

Authorized officer
J. Leitao
Telephone No.: (41-22) 338.83.38

004192126

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

To:
Sim & McBurney
Attn. STEWART, Michael, I.
330 University Avenue
6th Floor
Toronto, Ontario M5G 1R7
CANADA

Date of mailing *(day/month/year)* 17/10/2000	
Applicant's or agent's file reference 1038-1023MIS	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/CA 00/ 00207	International filing date *(day/month/year)* 29/02/2000
Applicant CONNAUGHT LABORATORIES LIMITED et al.	

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International S arching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Catherine Humbert

Form PCT/ISA/220 (July 1998)

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international pbulication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the languag of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international appplication is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
1038-1023MIS		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/CA 00/ 00207	29/02/2000	03/03/1999
Applicant		
CONNAUGHT LABORATORIES LIMITED et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[X] furnished subsequently to this Authority in written form.

[X] furnished subsequently to this Authority in computer readble form.

[X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PC[redacted] 00/00207

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/102 A61K39/116 A61K39/295 A61P31/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, PAJ, WPI Data, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BARENKAMP STEPHEN J ET AL: "Identification of a second family of high-molecular-weight adhesion proteins expressed by non-typable Haemophilus influenzae." MOLECULAR MICROBIOLOGY, vol. 19, no. 6, 1996, pages 1215-1223, XP000946619 ISSN: 0950-382X cited in the application page 1215, column 2, paragraph 1 page 1220, column 2 -page 1221, column 1, paragraph 1 --- -/--	1-4,7-9

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 October 2000	17/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Noë, V

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	S BARENKAMP: "Immunization with high-molecular weight adhesion proteins of nontypeable H. influenzae modifies experimental otitis media in chincillas" INFECTION AND IMMUNITY, AMERICAN SOCIETY OF MICROBIOLOGY, WASHINGTON, DC, US, vol. 64, no. 4, 1996, pages 1246-1251, XP002142962 ISSN: 0019-9567 cited in the application abstract page 1250 ---	1-3,7-9, 18,20,21
A	WO 94 21290 A (BARENKAMP STEPHEN J ;ST GEME JOSEPH WILLIAM III (US)) 29 September 1994 (1994-09-29) abstract page 2, line 1 - line 21 page 5, line 19 - line 26 page 6, line 29 - line 34 page 9, line 30 -page 10, line 9; example 1 page 23, line 7 - line 25 ---	1-3,7-9, 18-21
A	US 5 869 302 A (LOOSMORE SHEENA M ET AL) 9 February 1999 (1999-02-09) the whole document ---	1,5,6, 8-15
E	WO 00 35477 A (LOOSMORE SHEENA M ;CONNAUGHT LAB (CA); YANG YAN PING (CA); KLEIN M) 22 June 2000 (2000-06-22) cited in the application the whole document -----	1-27

INTERNATION EARCH REPORT

International application No.
PCT/CA 00/00207

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 27 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PC[redacted] 00/00207

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9421290 A	29-09-1994	AU 696207 B	03-09-1998
		AU 6400594 A	11-10-1994
		BR 9406589 A	30-01-1996
		EP 0689453 A	03-01-1996
		JP 11501003 T	26-01-1999
		US 5869065 A	09-02-1999
US 5869302 A	09-02-1999	US 5939297 A	17-08-1999
		US 5506139 A	09-04-1996
		AU 687619 B	26-02-1998
		AU 3337695 A	22-02-1996
		BR 9506272 A	12-08-1997
		CA 2171611 A	08-02-1996
		WO 9603506 A	08-02-1996
		EP 0729513 A	04-09-1996
		NZ 291750 A	24-10-1997
		US 6025342 A	15-02-2000
		US 6020183 A	01-02-2000
		US 6114125 A	05-09-2000
		CN 1136328 A	20-11-1996
		US 5665353 A	09-09-1997
		US 5935573 A	10-08-1999
		US 5656436 A	12-08-1997
		US 5981503 A	09-11-1999
		US 5962430 A	05-10-1999
WO 0035477 A	22-06-2000	AU 1543900 A	03-07-2000

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

STEWART, Michael I.
Sim & McBurney
330 University Avenue
6th floor
Toronto, Ontario M5G 1R7
CANADA

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	09.05.2001
Applicant's or agent's file reference: 1038-1023MS	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA00/00207	29/02/2000	03/03/1999

Applicant: CONNAUGHT LABORATORIES LIMITED et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Neumann, M Tel.+49 89 2399-7351

Form PCT/IPEA/416 (July 1992)